IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant: Gardner et al.	Confirmation No. 4887
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Docket No. UTSD:1510	Examiner: Noakes, Suzanne Marie
Title: NMR Detection of Foreign PAS Domain Ligands	

REPLY BRIEF

The Honorable Board of Appeals and Interferences
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Honorable Board:

We maintain that the Aug 06, 2008 Action does not establish that claims 3 and 5 are unpatentable under 35USC103(a).

Relevant background of the invention is provided in the Specification at p.1, line 22 – p.2, line 5:

> Some members of the PAS family are known to contain small molecules within their cores, allowing them to sense stimuli and regulate diverse biological processes. For example, heme binding by the PAS domains of FixL (Gong et al., 1998; Miyatake et al., 2000) and Dos (Delgado-Nixon et al., 2000) allows bacteria to sense oxygen levels; blue light photoreception in plant phototropins is achieved through a flavin molecule associated with their LOV domains (a PAS domain subclass) (Crosson et al., 2003); and binding of exogenous organic compounds by the C-terminal PAS domain of the aryl hydrocarbon receptor (AhR) displaces a chaperone protein, induces a conformational change and activates the transcription of xenobiotic metabolizing enzymes (Schmidt & Bradfield, 1996).

In all these examples, the cofactor is reportedly required for proper folding and functioning of the PAS domain within the context of the holo-protein.

However, for most PAS domains there is no evidence for such a cofactor. In fact, structurally characterized PAS domains without bound cofactors (Amezcua et al., 2002; Erbel et al., 2003; Morais Cabral et al., 1998) show tightly packed cores with no pre-formed cavities that would suggest a cofactor or ligand binding site.

Fesik (WO97/18471) discloses the use of particular two-dimensional $^{15}N/^{1}H$ NMR correlation spectra to identify ligand binding to target biomolecules. Fesik teaches nothing about PAS domains.

Edery (US 5,843,683) characterizes four PAS domain containing proteins (AHR, SIM, ARNT and PER) and use co-immunoprecipitation experiments to propose that PAS domains engage in PAS-PAS interactions. Edery proposes and claims assays for molecules that modulate PAS-PAS interactions.

Takahaski (US 6,291,429) describes circadian clock genes from humans and mice, and proposes contemplated uses of CLOCK polypeptides including use "in a screening assay for the identification of drugs or compounds that inhibit the action of CLOCK polypeptide (e.g., DNA binding)." Takahaski, col.9, lines 13-27.

Berkenstam (US 6,436,654) discloses and claims methods for identifying compounds which modulate the function of a functional domain of a variant of human HIF-1α that lacks at least one functional domain thereof.

Prior to our invention it was known to use NMR to identify ligand binding to target molecules. Prior to our invention it was known that PAS domains are protein interaction domains widely used for intra- and intermolecular associations. Prior to our invention it was known that there were two structurally and functionally distinct classes of PAS domains: one kind (e.g. PYP PAS) purified with a core-bound cofactor required for proper folding and formation, and crystallographic analysis showed the cofactor bound inside a core pocket; the

other class (e.g. HERG PAS) did not purify with a core-bound ligand, and crystallographic analysis revealed a tight core with no apparent core binding pocket. It is the latter class which is the subject of our claims, and we whole-heartedly agree with the Board's conclusion that the prior art suggests utilizing this class of PAS domains (without bound cofactors and showing tightly packed cores with no pre-formed cavities) for ligand screening:

> Takahaski suggests a method for identifying ligands for a PAS protein having a hydrophobic core (Answer 6). Edery also describes an assay method for identifying compounds that regulate a PAS domain protein's activity. Thus, despite the fact that these proteins have tightly packed cores with no pre-formed cavities - a fact that Appellants have not challenged - it was still suggested that these PAS domain proteins be utilized for ligand screening (see, e.g., Takahaski, at col. 9, ll. 14-16; Edery, at col. 46-50).
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> Decision, para. bridging p.6 and 7.

Prior to our invention the N-terminal PAS domain of the HERG potassium channel was postulated to self-regulate the HERG protein by binding the channel body, and we similarly whole-heartedly concur with the prior Board Decision's conclusion that it would have been obvious to try to target this activity with regulatory small molecules:

> The Specification refers to various prior art publications, including Morais Cabral *(Cell,* 95:649-655, 1998), for teaching "structurally characterized PAS domains without bound cofactors (... Morais Cabral et al., 1998) showing tightly packed cores with no pre-formed cavities that would suggest a cofactor or ligand binding site" (Spec. 2: 2-5). Morais Cabral, which Appellant admits satisfies the claim limitations for a PAS domain (App. Br. 5), compares the eag PAS domain of the HERG potassium channel to other PAS domain proteins known to comprise a ligand in their hydrophobic core *(see id.,* at 852, col. 2, describing the PYP photoreceptor which has a chromophore associated with its PAS domain). Morais Cabral conclude: "[g]iven the regulatory roles of PAS domains in other protein systems, we suspect that the eag domain will have a dynamic influence on the

gating of the HERG K+ channels through the binding of small molecule or protein effectors" *(id.,* at 854, col. 2). Thus, despite having a tightly packed core with no pre-formed cavity, in view of its similarity to other PAS domain proteins, Morais Cabral suggested that small molecules might regulate eag domain activity as they do for other PAS domains.

Decision, para. bridging p.7 and 8.

Our dispute lies in exactly what kind of screening assay the prior art suggests. We contend that the screening assays suggested by the prior art are not what we are claiming.

For example, Morais Cabral used scanning mutagenesis to identify a hydrophobic patch on the surface of the HERG PAS domain that forms an interface with the body of the potassium channel to which it tightly binds. To do this, they introduced various point mutations <u>on the surface</u> of the PAS domain and then assessed their functional impact by monitoring channel deactivation in electrophysiological recordings of Xenopus oocytes expressing the mutant proteins. Id., Fig. 5B-C. Morais Cabral found that the presence of the PAS domain bound to the channel via that surface patch slows the rate of channel deactivation, suggesting a regulatory function. Id., Abstract; p.649, col.2, first full para.; p.652, Fig. 5A and first and second full paragraphs. Morais Cabral's regulation can visualized by imagining the red PAS domain of Fig. 1A swinging up and binding (via its hydrophobic surface patch – green in Fig. 5A) to the blue channel core. What Morais Cabral is suggesting is that small molecules or effector proteins might target and interfere with or regulate this intramolecular PAS-channel binding. To the extent this suggests a screening assay to identify foreign small molecule regulators, Morais Cabral provides a functional electrophysiological assay for doing just that. Instead of screening regulatory point mutations, the same methods could be used to screen for regulatory small molecules.

The cited Edery (US 5,843,683) and Takahaski (US 6,291,429) similarly propose screening compounds in functional assays. In particular, Edery proposes in vivo, transcriptional reporter assays for PAS dimerization:

> In the third aspect, the invention features an in vivo assay method for

identifying, screening and characterizing compounds potentially useful for treatment of diseases or disorders arising from abnormal PAS-PAS binding affinities. The method includes transfecting purified nucleic acid encoding a PAS-containing protein into a host cell which contains, or is manipulated to contain nucleic acid of a reporter gene whose transcription is regulated by the presence or absence of dimerized PAS-containing proteins. Test sample compounds can then be introduced and the effect on reporter gene transcription assayed.
Edery, col.3, lines 46-50.

The cited Berkenstam (US 6,436,654) similarly discloses methods for identifying compounds which modulate the "function" of HIF-1α, and the only specifically disclosed assay is a transcriptional reporter read-out assay. Berkenstam, Figs. 4-13; Example 5 at col. 13-14.

And the cited Takahaski proposes similarly inhibiting functional activity of the CLOCK polypeptide, such as DNA binding:

A CLOCK polypeptide of the present invention has numerous uses. By way of example, such a polypeptide can be used in a screening assay for the identification of drugs or compounds that inhibit the action of CLOCK polypeptide (e.g., DNA binding). The CLOCK polypeptide is an integral component of the circadian clock of mammals. As set forth below, animals lacking the ability to produce the CLOCK polypeptide have significant dysfunctions in their circadian clock. Mutant animals producing an altered CLOCK polypeptide can be given the normal CLOCK polypeptide together with suspected agonists or antagonists and the effects of such treatment on the restoration of a normal circadian rhythm can be determined. The CLOCK polypeptide can also be used to treat animals having circadian rhythm dysfunctions as set forth hereinafter. Takahaski, col.9, lines 13-27.

No where do any of these references suggest screening for small molecules that simply bind the subject PAS domains. A small molecule that simply bound the subject PAS domain

would be of no interest to the authors of the cited art. Morais Cabral expresses no interest in identifying a small molecule that simply binds the HERG PAS domain; rather, all that Cabral is interested in, and all that his assay will even detect, are compounds which interfere with channel activation as measured in his electrophysiological assays. Hence, we can not agree that these references suggest screening for compounds in an in vitro binding assay format, such as NMR.

In any event, even if such as suggestion is inferred, it does not reach our claims. For example, to the extent Morais Cabral was interested in PAS binding compounds, he would be interested in compounds which bound the hydrophobic surface patch interface and thereby had the potential to interfere with its function. No where does Morais Cabral or any of these references suggest targeting the hydrophobic core of the subject PAS domains. The crystal structures of these PAS domains show a tight core with no apparent binding cavity. The 15 point mutations Morais Cabral analyses were chosen to be "spread over the domain surface" (p.651, first full para; Fig. 5A) – not within the interior of the domain, which Morais Cabral had no reason to target. None of Morais Cabral, Takahaski, Berkenstam, or Edery had or offers any reason to suspect the tightly-packed core of their respective PAS domain could bind small molecules, or if it could, that such binding would induce surface changes that would in turn impact the domain's function in a manner relevant to their functional assays.

Our claims are specifically directed to a method of detecting binding of a PAS domain of a protein with a foreign core ligand of the PAS domain, wherein the PAS domain is prefolded in its native state. The method specifically requires the steps of: (a) determining from NMR analysis of the PAS domain that the PAS domain comprises a hydrophobic core that has no NMR-apparent a priori formed ligand cavity; (b) detecting a first NMR spectrum of the PAS domain in the presence of a foreign ligand; (c) comparing the first NMR spectrum with a second NMR spectrum of the PAS domain in the absence of the ligand; and (d) determining the presence of the ligand specifically bound within the hydrophobic core of the PAS domain. The cited art does not suggest applying this particular screening assay to PAS domains with a hydrophobic core having no NMR-apparent a priori formed ligand cavity. There is no motivation in the cited art to specifically assay core ligand binding as claimed.

In a particular embodiment, the protein consists of the PAS domain. Specification, p.5,

line 22; claim 5. Here, there is no other domain to participate in any intra- or inter-molecular interaction, further distinguishing cited art which rely on functional interaction with non-PAS domains.

Though the cited art does not support a prima facie case for obviousness, for good measure we provided affirmative evidence documenting the fact that one skilled in the art would have considered the claimed invention nonobvious at the time it was made (see, Declarations of Professors Stephen R. Sprang and Kevin H. Gardner).

We maintain that the screening assays suggested by the prior art are not what we are claiming. We appreciate that we are asking the Board, on this record, to slightly modify a statement in the prior Decision (para. bridging p.6 and 7), which we believe went beyond the stated rationale:

> We do not agree that "one skilled in the art would not have suspected that ... PAS domains (without known cofactors and having tightly packed cores with no pre-formed cavities that would suggest a cofactor or ligand binding site) would be rational candidates to screen for ~~core~~ ligand binding" (Appeal Br. 5). Takahaski suggests a method for identifying ligands for a PAS protein having a hydrophobic core (Answer 6). Edery also describes an assay method for identifying compounds that regulate a PAS domain protein's activity. Thus, despite the fact that these proteins have tightly packed cores with no pre-formed cavities - a fact that Appellants have not challenged - it was still suggested that these PAS domain proteins be utilized for ligand screening (see, e.g., Takahaski, at col. 9,11. 14-16; Edery, at col. 46-50). [strike-through added].

We believe the Board's statement of facts and reasoning supports the conclusion, with which we agree, that PAS domains (without known cofactors and having tightly packed cores with no pre-formed cavities that would suggest a cofactor or ligand binding site) would have been rational candidates to screen for ligand binding. Our only point of contention, detailed above, is whether the specifically clamed hydrophobic core binding assay in this particular subset of PAS domains was suggested.

Respectfully submitted,
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